The Effect of Hyperthermia on Intracellular Sodium Concentration in Isolated Human Cells. A Preliminary Report.

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Abstract

Groups of human squamous epithelial cells labeled with the sodium sensitive fluorescent dye, Sodium Green (Molecular Probes, OR) were subjected to a 20 minute hyperthermic stress at temperatures as high as 50^{o}C and then cooled to 37^{o}C. Changes in fluorescence were determined at one minute intervals using an interactive laser cytometer. Cells raised to temperatures in excess of 43^{o}C showed a significant rise in fluorescence and thus a rise in intracellular sodium concentration, $[Na^{+}]_{i}$. Upon return to 37^{o}C, $[Na^{+}]_{i}$ in these cells did not fall but continued to rise at an increased rate compared to controls.

1. Introduction

Heat illnesses have played an important role in the outcome of many military campaigns and promises to increase in significance as more and more units deploy to hot climates.

The symptoms of heatstroke are well known; however, the pathophysiological mechanisms responsible for those symptoms and possible death are poorly understood. The early and important subcellular alterations that ultimately cause dyshomeostasis and organismic heatstroke are virtually unknown.

Hubbard's "energy depletion mechanism" of heatstroke predicts a rise in internal sodium concentration (Hubbard, 1990), but this has not been clearly demonstrated. In this study, we found elevations in $[Na^{+}]_{i}$ in human squamous epithelial cells at elevated temperatures, by quantifying changes in the fluorescence of an internalized Na^{+}-sensitive fluorophore.

2. Materials and Methods

2.1 Cells

Human squamous epithelial cells were suspended in Hanks' Balanced Salt Solution (HBSS) containing 5 µm Sodium Green at pH 7.4, incubated for 30 minute at 25^{o}C, and rinsed with fresh HBSS. The cells were then transferred to a temperature controlled optical chamber and allowed to stabilize at 37^{o}C for 30 minutes before beginning the experiment.

2.2 Fluorescence

Finely focused pulses of low energy laser radiation (488 nM) were directed through a microscope across the surface of a labeled cell which is then scanned completely (ACAS 570, Meridian Inst. Co., Okemos, MI). Cell fluorescence was detected by a photomultiplier, stored within an internal computer and rendered as a 2-dimensional pseudo-color image, with the color related to fluorescence intensity. Laser energy was adjusted to minimize bleaching of the fluorophore within the cells (<2% photobleaching after 60 scans). Absolute

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intracellular calibration of the fluorophore is technically difficult, and only changes in normalized fluorescence are reported. As an additional control, the fluorescence of the Sodium Green free acid was determined in HBSS at various temperatures.

Cells (n>14) were placed into groups to be heated from 37°C to different final temperatures. Laser scans of the cells were conducted once a minute at 37°C for 10 minutes, raised to a target temperature, maintained at that temperature for 20 minutes, cooled back to 37°C , and maintained at 37°C for 10 min. The total duration of the experiment was less than one hour. Control cells were maintained at 37°C for one hour while scanned as above.

3. Results

Cell shape and size remained constant throughout the experiment. Unlabeled cells show very low or undetectable levels of fluorescence, while labeled cells at 37°C, showed a strong fluorescence with a small rise in mean fluorescence over time (drift) (Fig. 1). Raising the temperature caused an immediate transient decrease in fluorescence ($p<0.05$, Fig. 1, Arrow) followed by a continual, gradual rise. At 42 and 43°C, the slope was not constant but gradually increased. Cooling back to 37°C did not result in a fall in fluorescence; instead, at temperatures ≥ 43°C, the fluorescence continued to rise even further.

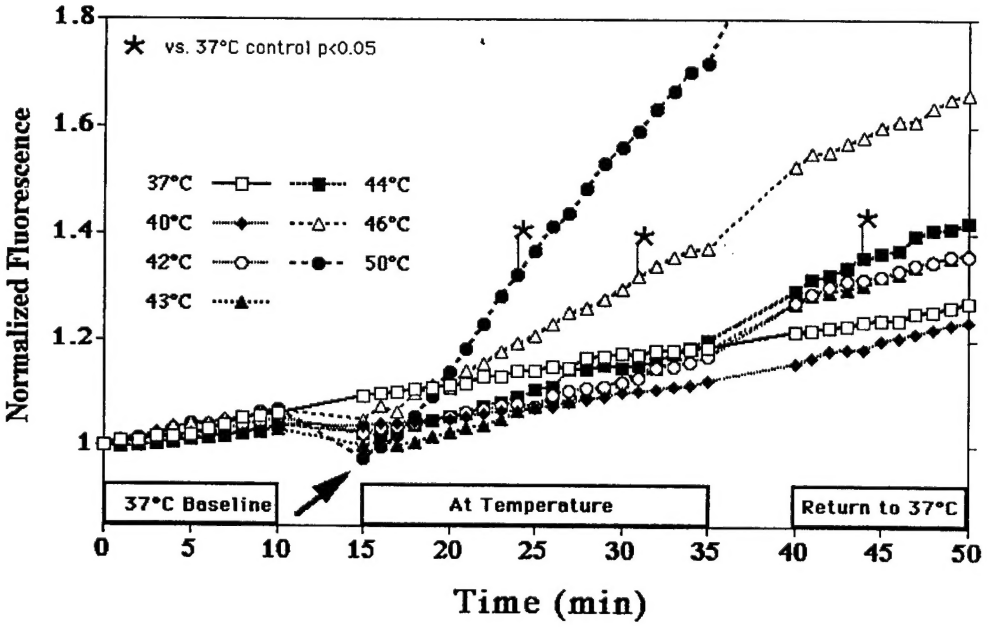

Figure 1. Fluorescence vs. Time. Groups of human squamous epithelial cells *in vitro* were labeled with a sodium-sensitive dye (Sodium Green), then heated to the indicated temperatures, or held at 37°C as controls. Fluorescence showed a small upward drift during the 37°C baseline. Upon heating, cellular fluorescence first showed a transient fall (Arrow) followed by a rise. Upon cooling to 37°C, the fluorescence did not return to baseline, but increased still further.

4. Discussion

Previous studies on heatstroke in animals and humans reported little or no change in plasma Na^+ concentrations, suggesting no significant changes occurred in $[Na^+]_i$ (Gaffin et al. 1994). Those studies utilized less-sensitive analytical techniques and indirect methods in attempting to determine a necessarily small intracellular change compared to a high extracellular $[Na^+]$ background (Yi et al. 1983). The ACAS system employed here is markedly more sensitive to local changes in Na^+, since it expresses Na^+ concentrations as a change in relative fluorescence within individual cells against a very low background.

4.1 Heating

$[Na^+]_i$ depends upon the relationship between the rates of Na^+ influx and efflux. In a steady-state influx and efflux are balanced and the $[Na^+]_i$ remains constant. Hyperthermia increases the rate of Na^+ influx.

At 37°C and normal ionic concentrations, Na^+ efflux is controlled almost exclusively by the Na^+-K^+-ATPase pump. Its pumping rate increases in response to a rise in temperature and/or a rise in $[Na^+]_i$. Therefore, moderate rises in temperature, causes an increase in Na^+ influx and efflux. The initial drop in $[Na^+]_i$ upon heating (Fig. 1, Arrow) can occur if the Na^+ efflux is greater than its influx. The eventual elevated slope of fluorescence suggests that Na^+ influx rapidly becomes greater that its efflux. Above 44°C, the upwardly curving slope of fluorescence suggests a gradual thermal inactivation of the Na^+-K^+-ATPase pump.

4.2 Cooling

Other groups have shown some activity of the Na^+-K^+-ATPase pump at temperatures as high as 45°C. If this is so, then cooling back to 37°C from 44°C should cause little change in Na^+ influx compared to baseline values. At higher temperatures and if the Na^+-K^+-ATPase pump was thermally inactivated prior to cooling back to 37°C, then the rate of rise of $[Na^+]_i$ should be much higher than its 37°C baseline value because the Na^+ pump has been denatured. This is seen in Figure 1 at 50°C.

A small increase in $[Na^+]_i$ can lead to a large rise in $[Ca^{++}]_i$ influx via the Na^+/Ca^{++} exchanger since the Ca^{++} driving force depends upon the third power of $[Na^+]_i$ (Reeves et al. 1994). Therefore, heating can be expected to eventually activate Ca^{++}-dependent pathways, possibly leading to the production of immune modulators (Gathiram et al. 1987; Bouchama et al. 1993; Chang, 1993).

4.3 Energy-Depletion Model

In the energy depletion model elevated temperatures increases the rate of the Na^+-K^+-ATPase pump, with increased utilization of ATP resulting in energy loss and internal heat production. This would speed up the pump still further in a vicious circle, until its maximum rate is reached. Acidosis by 0.1-0.2 pH is a consequence of severe hyperthermia. In this case the Na^+-H^+ exchanger would be activated to lower the intracellular H^+ concentration, and, if the temperature is near the maximum rate of the Na^+ pump, intracellular Na^+ concentration would rise, thus accounting for the high rate of rise in Na^+ seen at highest temperatures.

If nerve cells behave similarly then the rise in $[Na^+]_i$ described here would, according to the Nernst equation, reduce the magnitude of action potentials. This could then affect physiological mechanisms, psychological function and physical coordination, and contribute to the pathophysiology of heatstroke.

In summary, heating caused an elevation in $[Na^+]_i$ in human squamous epithelial cells that was not rapidly reversed with cooling.